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13. Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information) The p53 and pRb tumor suppressor pathways are frequently altered in human breast cancer. Although animal models have begun to explore mechanisms for these proteins, the roles can be different depending on the cancer type. Our previous studies in a mouse brain epithelial tumor model have demonstrated the importance of pRb in tumor initiation and of p53 in tumor progression, and have established p53-dependent apoptosis as a means of tumor suppression. In this model, brain cells are induced to proliferate aberrantly by tissue-specific expression of T ¹²¹ , a small T antigen oncoprotein that inactivates pRb. This causes slow growing, but highly apoptotic tumors. Further inactivation of p53 causes a dramatic decline in cell death and rapid acceleration of tumor growth. We propose similar studies to examine the pRb and p53 roles in breast cancer. The full T antigen oncoprotein (inactivates both pRb and p53) has been shown to induce mammary tumors in transgenic mice. Here, the T ¹²¹ oncoprotein will be tissue-specifically expressed in mammary epithelium by mammary-specific promoters to test the role of pRb. Further analysis using knock out strains will address the role of p53. Such preclinical models are essential for progress in breast cancer research.				
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Introduction

An understanding of how breast cancer develops and the design and testing of innovative therapies will require the establishment of preclinical animal models. This is because of the distinct changes in breast cells that lead to this cancer cause certain biological changes that arise within a very specific tissue environment. We are fortunate that technologies exist to introduce specific genetic changes into the experimental mouse. This allows us to determine the impact of genetic changes observed in human cancer in the context of the whole organism. The pRb tumor suppressor pathway is altered in a large fraction of human breast cancers. Yet, the impact of pRb inactivation on mammary tissue has not been tested before. We will determine the effect on mammary tissue after inactivation pRb proteins by tissue specific expression of viral oncoproteins, T₁₂₁. We will further test the role of the p53 tumor suppressor – a gene that is also altered in about 50% of human breast cancers. This will be done by breeding the mice with mammary-specific pRb inactivation to p53-deficient mice. Our preliminary work suggests that these experiments have every chance for success in producing a valued preclinical model of breast cancer.

Body

Original Specific aims

We have explored the function of pRb and p53 in several sites within the animal using tissue-specific promoters to express wild-type and mutant T-Ags combined with the use of knock-out mice. Here, using the same approach, we will determine tumorigenesis mechanisms in mammary epithelium (ME). Our original specific aims were as follows:

- **I. To determine the impact of pRb-inactivation in mouse mammary epithelium.** The T₁₂₁ oncoprotein will be expressed in ME using the MMTV and WAP promoters. Several lines of transgenic mice (TgME-T₁₂₁) will be generated and examined for mammary-specific phenotypes. Cell cycle activity and apoptosis indexes will be determined relative to non-transgenic littermates in virgin and lactating mammary glands. Our preliminary studies indicate that T₁₂₁ will elicit an abnormal phenotype in ME.

- **II. To determine the role of p53 in mammary tumor suppression subsequent to pRb inactivation.**

TgME-T₁₂₁ mice will be crossed with p53^{-/-} mice to generate TgME-T₁₂₁ p53^{+/-} and TgME-T₁₂₁ p53^{-/-} mice. Tumor incidence will be assessed and ME apoptosis and proliferation will be measured. If tumors ensue, tumor progression will be analyzed at the molecular, cellular and morphological levels.

Progress

In last year's progress report we described the WAP--T₁₂₁ founder mice and lines that had been generated. These are summarized in the table below for reference. In the past year we have made substantial progress toward our initial goals. We previously noted that female mice in line 6 were beginning to develop mammary gland tumors at around 13 months of age after several pregnancies (required to induce the WAP promoter). We have now characterized a substantial number of mice and have shown that these animals develop adenocarcinoma with 100 % penetrance.

We have now generated TgWAP-T₁₂₁ p53^{+/-} mice and have shown that the tumor latency is reduced to 7 months. We have also shown that tumor growth rates are accelerated in this background and that tumors undergo loss of the wild type allele 80 % of the time. A significant amount of the apoptosis induced by T₁₂₁ in the mammary gland early in life was shown to be dependent on p53, and heterozygosity at p53 also caused a reduction in apoptosis. These results are presented in the figures below.

Table 1. Summary of TgWAPT121 Transgenic Mice

Transgenic Line	Expression		Mammary Gland	
	Tissue	Protein	Gross Phenotype	Abnormalities
TgWAPT121-0	N.D	N.D	death ²	N.D
TgWAPT121-1	N.D	N.D	no line, death ³	N.D
TgWAPT121-2	lactating MG	++	normal	HAP, HM,
TgWAPT121-3	lactating MG	+++	normal	HAP, HM,
TgWAPT121-6	lactating MG	++++	not nursing	HAP, HP, SL,
HM, tumors				
TgWAPT121-7	lactating MG, brain, kidney	++++	not nursing, death ¹	HAP, HP, SL, HM
TgWAPT121-8	N.D	N.D	no line, death ³	N.D

(MG): mammary gland; (N.D): not determined; (HAP): high apoptotic bodies; (HP): high proliferation; (SL): small sized mammary lobules; (HM): high mitotic figures.

¹~50% mice died of unknown causes.

²All mice died at 1 to 1.5 month old.

³Founder died at 1 month old, no offspring.

We are currently analyzing the progression of tumors in these mice to determine the full mechanism of p53 tumor suppression. We are also analyzing TgWAP-T₁₂₁ p53^{-/-} mice; however, these mice die of thymic lymphoma at around 5 months of age precluding analysis beyond this time. We are therefore performing mammary gland transplants to pursue these studies further.

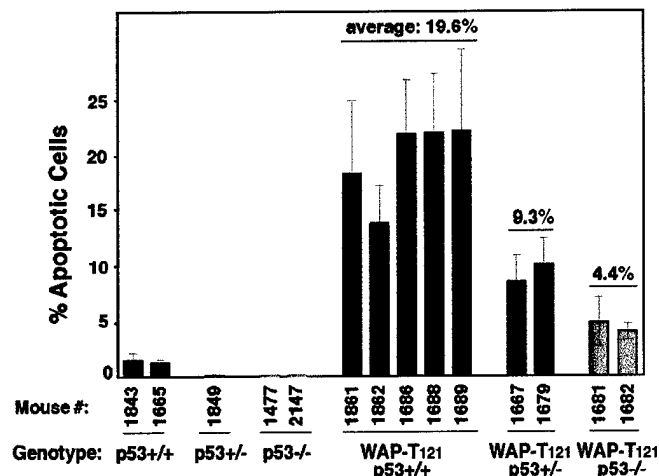


Figure 1. Apoptosis induced by Rb inactivation is p53-dependent. The apoptotic index as detected by TUNEL is shown for the mammary glands of mice with indicated genotypes. The average apoptotic index is indicated where significant levels of TUNEL-positive cells were detected.

III. Future Plans

We will continue to breed to generate TgWAP-T₁₂₁ p53^{+/-} and TgWAP-T₁₂₁ p53^{-/-} mice for mechanistic studies on tumorigenesis mechanisms. A timed series of tissues will be analyzed for apoptosis and proliferation rates. Tumor progression will be examined by analyzing the levels of genetic instability and angiogenesis. Similar studies will be performed in transplanted TgWAP-T₁₂₁ p53^{-/-} glands.

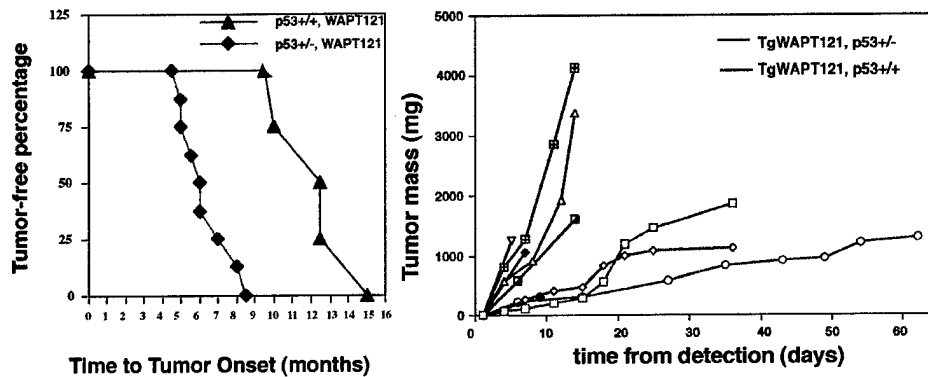


Figure 2. Survival time (left) and mammary adenocarcinoma growth rates (right) depend on p53 status. Times to sacrifice due to mammary adenocarcinoma are indicated for TgWAP T₁₂₁ p53^{+/-} (red) and For TgWAP T₁₂₁ p53^{+/+} (blue). The graph on the right shows mammary tumor growth rates as measured over time in the same mouse using a caliper.

Key Research Accomplishments

1. We have established a model of mammary cancer that is based on genetic aberrations frequently observed in human breast cancer.
2. We have shown that inactivation of the Rb pathway elicits similar responses in multiple cell types.
3. We have demonstrated that apoptosis induced by Rb inactivation is largely dependent on p53, and that the level of apoptosis is dose-dependent with respect to p53.
4. We have shown that heterozygosity at the p53 locus accelerates the development of mammary tumors thus providing a system by which to study tumor progression.

Reportable Outcomes

Much of the above list of accomplishments is included in a manuscript in preparation for publication.

Conclusions

Stated in research accomplishments above.

References

Wu, H. Simin, K., Cardiff, R. and T. Van Dyke (2001) "Specific inactivation of the Rb pathway in mammary epithelium predisposes to adenocarcinoma that is accelerated by p53 heterozygosity" in preparation.

Appendices

None.